II. RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 1-3 and 5-10, 13-15 and 18-20 are amended in the attached amendment and claims 21- 60 are added in the attached amendment. Claims 1- 60 are therefor pending. For the convenience of the Examiner, the claims as they will appear after entry of the amendments contained herein are set forth in Appendix 1.

B. New Matter is not Introduced

New claims 21 - 24 and 26 - 37 were initially filed in the co-pending parent application No. 08/726,211, filed 10/04/1996 as claims 31-37, 39-41, 48-50, 52-54 and 56 and therefore do not introduce new matter. These claims are supported by the instant specification. Claims 21 - 24 are supported at, for example, page 5, line 17-18 and page 40, line 5 - 19. Support for claims 25-37 can be found at page 4 line 7 to page 6 line 8 and page 11 line 2 to page 24 line 2.

Claims 1 and 9 were amended to exclude the negative limitation and to clarify the nature of the invention as being drawn to compositions of neutrally-charged lipid associations containing neutral lipids but not necessarily excluding cationic lipids. Support for this amendment can be found on page 6, line 14.

Claims 18-20 were amended to more clearly state the invention.

New claims 25 and 38-60 do not add new matter. Support for these claims can be found throughout the specification. Support for P-ethoxy polynucleotides can be found at, for example, page 14, lines 25-29. The non-specific toxicity of the methods in claim 38 and 49 is discussed in the application as shown in FIG. 2 and on page 34, lines 6-10. "Jurkat, Raji and Daudi cells were also treated with L-control oligos and empty liposomes. Non-specific toxicity could be

observed when greater than 6 μmol/L of L-OS were used, but not with empty liposomes" Support for the ionic lipid DMPC is found on page 21, line 27.

C. Benefits of Priority are Claimed

The Action first notes that reference to the prior application to which this application claims priority as well as the current status of all nonprovisional parent applications must be inserted in the application under 35 U.S.C. 119(e) or 120. Applicants have added this information in the attached amendment.

D. The Oath/Declaration is Corrected

The Action next notes that the oath or declaration is defective. The Examiner states that non-initialed changes were made to the address of inventor Tormo. A new declaration in compliance with 37 CFR 1.67(a) is submitted herewith. Applicants respectfully request that this rejection be withdrawn.

E. Double Patenting is Avoided

The Examiner states that Claims 1-20 conflict with claims 1-41, 43-50, and 52-56 of co-pending application No. 08/726,211, and are provisionally rejected under the judicially created doctrine of double patenting. The provisional double patenting of claims 1-9 is overcome by the cancellation of claims 1-9 in application No. 08/726,211 in an amendment filed July 10, 2000, attached herein as Exhibit A. Applicants will remove the issue of double patenting for claims 10-20 when the provisional double patenting rejection becomes a double patenting rejection upon the issuance of either the current application or application No. 08/726,211 by filing a terminal disclaimer.

F. The Claims are Definite

The Examiner states that claims 1-20 are indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. Claims 1, 9 and 10 are rejected as indefinite due to the recitation "a second, bcl-2 encoding polynucleotide". Claims 2-8 and 11-20 are rejected as indefinite due to dependence on claims 1 or 10. Claims 1-3, 6, 9, 10, 13 and 15 have been amended to clarify the polynucleotides discussed in the claims; the "first" and "second" designations for the polynucleotides have been removed. In claims 1-3, 6 and 9 the qualifier "P-ethoxy" was added to distinguish the first listed polynucleotide from the "second," or Bcl-2-encoding polynucleotide and to more clearly claim the invention. Support for P-ethoxy polynucleotides can be found at, for example, page 14, lines 25-29. In claims 10, 13 and 15 , the qualifier "antisense" was added to distinguish the first listed polynucleotide from the "second," or Bcl-2-encoding polynucleotide. Support for this amendment can be found, for example, on page 5, line 25, and page 11 line 3.

The Examiner next rejects claims 5-8 as indefinite due to the recitation "the lipid" because it refers back to claim 1, which recites two different lipids and it is unclear which lipid "the lipid" is referring to. Claims 5, 7-8 and 14 have been amended to clarify the claimed lipid as the neutral lipid.

Next, the Examiner rejects claim 9 as indefinite due to the recitation "is active" because it is unclear what activities the phrase "is active" is meant to impart to the promoter. The claim has been amended and to recite that the promoter is capable of expression in the target cell. Support for this amendment is found on page 16, lines 15-16.

The Action next asserts that the methods of claims 10-20, drawn to methods of inhibiting the expression of a gene, are incomplete as there is no step in each method which relates back to the outcome set forth in the preamble. Claim 10 has been amended and now relates back to the preamble, "a method of inhibiting a Bcl-2-associated disease" with the phrase "and administering said association to a cell ... thereby inhibiting growth of said cell."

G. The Claims are Enabled

Claims 10-20 are rejected under 35 U.S.C. 112 first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and use the invention.

The Examiner argues that there are no examples presented which demonstrate an inhibition of a disease state and that there is no correlation between a reduction of proliferation of injected lymphoma cells with an effective treatment for bcl-2 associated disease. The Action then asserts that it would require undue trial and error experimentation for one skilled in the art to practice the methods of inhibiting a disease using a composition of a neutral lipid and a bcl-2 targeted antisense oligonucleotide as claimed in claims 10-20.

Applicants respectfully traverse, and point out that the specification, on page 35, lines 24-29 states:

> [T]he inhibition of Bcl-2 protein leads to cell growth inhibition in cells that are dependent on the presence of Bcl-2 protein for maintaining viability. Gene transfer experiments have provided evidence that Bcl-2 plays an important role in maintaining lymphoid cell survival *in vitro*, although other autocrine growth factors may also be involved (Vaux *et. al*, 1988; Reed *et. al*, 1990b; Blagosklonny and Neckers, 1995).

Thus, a correlation between treatment for bcl-2 associated disease and proliferation of injected lymphoma cells is taught. Similarly, example 3 demonstrates treatment of cancer cells and examples 5 - 7 demonstrates how to test *in vivo* inhibition of a disease state. A declaration of Tari and Lopez-Berestein presented in pending U.S. Application No 08/726,211, filed 10/4/1996, a copy of which is included as Exhibit B demonstrates the correlation between *in vitro* studies and *in vivo* inhibition of disease state. Applicants also remind the Examiner that use of examples are not required.

In Vitro Results Correlate with In Vivo Inhibition of Disease

The Examiner asserts that, because the examples show an unpredictability in the *in vitro* treatment of cancer cells, the specification does not provide guidance for determining what cell lines would respond *in vivo* or *in vitro*. The Examiner also argues that there is no evidence that inhibition of a cancer cell line *in vitro* would correlate with the inhibition of a disease state *in vivo*.

Applicants contend that the specification is fully enabling for *in vivo* methods. Example 3 demonstrates antisense technology for the *in vitro* treatment of cancer cells for four separate cell lines, giving guidance for determining what cell lines would respond *in vitro*. The instant application also teaches how to use antisense technology for treating cancer at page 5, the paragraph beginning on line 7, including treatment in a human. For example, the composition may be delivered to a human patient in a volume of 0.50-10.0 ml per dose or in an amount of 5-30 mg polynucleotide per m^2. The composition may also be delivered 3 times per week for 8 weeks.

Applicants submit that the issues raised in this rejection are the same as described in *In re Brana*, 51 F.3d 1560, 1566, 34 U.S.P.Q.2d 1436, 1440-43 (Fed. Cir. 1995) where a pharmaceutical composition was rejected under 35 U.S.C. § 112. The composition in *Brana* was rejected because 1) of a failure to describe any specific disease against which the claimed compounds were active, and 2) the tests in the prior art and the Specification were not sufficient to establish a reasonable expectation that the claimed compounds had a practical utility (*i.e.*, antitumor activity in humans). In regards to the second rejection the Commissioner argued that:

> Such *in vivo* tests in animals are only preclinical tests to determine whether a compound is suitable for processing in the second stage of testing, by which he apparently means *in vivo* testing in humans,

> **and therefore are not reasonably predictive of the success of the claimed compounds for treating cancer in humans."**

In re Brana, 51 F.3d at 1566, 34 USPQ2d at 1442 (Emphasis added).

The Federal Circuit rebuked the Office for requiring this higher standard for proof of therapeutic utility, stating:

> The Commissioner, as did the Board, **confuses the requirements under the law for obtaining a patent with the requirements under the law for obtaining government approval to market a particular drug for human consumption**. See *Scott* v. *Finney*, 34 F.3d 1058, 1063, 32 USPQ2d 1115, 1120 (Fed . Cir. 1994) ("Testing for the full safety and effectiveness of a prosthetic device is more properly left to the Food and Drug Administration (FDA). **Title 35 does not demand that such human testing occur within the confines of the Patent and Trademark Office (PTO) proceedings.**"

In re Brana, 51 F.3d at 1566, 34 USPQ2d at 1442 (Emphasis added) .

Applicants' further submit that the Examiner is applying an even higher standard of enablement than that established in *In re Brana*, asserting in effect that the murine tests "are not reasonably predictive of the success of the claimed compounds" in humans.

Further, proof that *in vitro* studies correlate with the inhibition of a disease state *in vivo* is included in a declaration of Tari and Lopez-Berestein presented in pending U.S. Application No 08/726,211, filed 10/4/1996 and included as Exhibit B. This declaration describes *in vivo* studies that were done using the compositions and methods of the claimed invention. Mouse studies conducted in the laboratory of the inventors demonstrate that the inventors observed definite morphological differences in tumor bearing mice treated with liposomal anti-Bcl-2 oligonucleotides compared to untreated or control treated mice. This data shows that there is a reasonable correlation between the described activity and the asserted utility. Therefore, the Patent Office should withdraw this rejection.

The Action expresses a concern with the unpredictability of using *in vivo* antisense due to target accessibility and delivery issues, as discussed in Agrawal, Branch and Crooke, stating that delivery of antisense oligonucleotide targeted to bcl-2 *in vitro* would not provide guidance for delivery of an antisense targeted to bcl-2 *in vivo*. Applicants direct the Examiner's attention to Examples 5 - 7 which provide protocols for *in vivo* testing, such as tests using mouse models, clinical trials and human treatment. Delivery systems for *in vivo* delivery are taught in the specification, beginning at page 21 line 16-19 where it states that

> The lipid may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression vectors have been successfully employed in transfer and expression of a polynucleotide *in vitro* and *in vivo*, then they are applicable for the present invention.

Alternative delivery systems including retroviruses, adenoviruses, other viral vectors and non viral methods are discussed beginning at page 24. These systems either can be used for *in vivo* delivery or can be adapted for *in vivo* use.

The Mouse Models is Appropriate

The Examiner asserts that immunosuppressed mouse models do not always correlate with therapeutic results in humans or other organisms, citing Gura and Golden. The Examiner also asserts that there is no art recognized nexus disclosed between the mouse model presented and a disease state nor any evidence that results obtained from the mouse model would extend to other organisms, cell lines, neutral lipid compositions, or blc-2 targeted oligonucleotides. Applicants maintain that the mouse model is a reliable predictor of *in vivo* efficacy in treating humans afflicted with the various cancers. Examples 5-7 provide protocols for *in vivo* testing, such as tests using mouse models, clinical trials and human treatment.

Applicants maintain that there are sufficient teachings of the efficacy of the mouse model in the instant situation. Dr. Richard, who presented a declaration in pending U.S. Application No 08/726,211, filed 10/4/1996 included as Exhibit C herewith, states

> Nude and SCID mice data are often predictive of responses in human trials. This confirms that one could use the methods, compositions and kits described in the specification to treat patients having a BCL-2 associated disease. It is my opinion that, a review of the mice data in the previous declaration provides a practitioner sufficient guidance to prepare a protocol to practice the claimed invention.

Further, with regard to the Examiner's position that the nude mouse model is not predictive of results of normal patients, Applicants contend that the nude mouse has been used in experimental and clinical research since it was first described in 1969 (Rygaard and Povlsen, 1969), attached as Exhibit 2. It is generally accepted that the nude mouse model is the best indication of what can be expected from human trials as supported by numerous studies finding that transplants of human tumors into the nude mouse provide an accepted model for testing the clinical efficacy of anticancer agents (Inoue *et al.*, 1983; attached as Exhibit 3, Guiliani *et al.*, 1981; attached as Exhibit 4, Giovanella *et al.*, 1983; attached as Exhibit 5, Tashiro *et al.*, 1989, attached as Exhibit 6; Khleif and Curt in *Cancer Medicine,* 4th Ed., pp. 855-68, 1997, attached as Exhibit 8). These studies demonstrate that the mouse model emulates the clinical situation in a number of diseases and cancers including lung, breast, and ovarian cancers. Furthermore, predictions from the nude mouse model studies also correlate well with clinical studies.

The nude mouse model has also been used to screen for, study and confirm anticancer effects of numerous agents. For example, doses of compounds used in preclinical animal studies can often be correlated to studies in human clinical trials (Tashiro *et al.*, 1989, attached as Exhibit 6). Additionally, correlation between the nude mouse and human clinical responses to cyclophosphamide, 1-(4-amino-2-methylpyrimidin-5-yl)-methyl-3-(2-chloroethyl)-3-nitrosurea

hydrochloride, vinblastine and 5 fluorouracil have been shown. Other studies have employed BALB/c nude mouse model for evaluating the antitumor activity for human breast cancer treatment of a variety of drugs, including vincristine, vinblastine vindesine, dauonomycin, mitoxantrone, and 5 fluorouracil amongst others (Inoue *et al.*, 1983, attached as Exhibit 3). These studies showed good correlation between the anticancer activity of various drugs in the nude mouse model for human breast cancer and in clinical treatment in humans.

In yet another comprehensive study (Guiliani *et al.*, 1981, attached as Exhibit 4), BALB/c nude mice were transplanted with breast, colon, lung, melanoma, ovarian prostate and larynx cancers and the effects of doxorubicin on these cancer models was studied. It was found that in each case the results from the model studies correlated extremely well with clinical data. The National Cancer Institute has even employed a development scheme in assaying for *in vivo* antitumor activity in which the human tumor cell line most sensitive to an active candidate *in vitro* is tested as a xenograft in a subcutaneous implant site in a nude mouse (*Cancer: Principles & Practice of Oncology,* 5th Ed., 1997, pp. 392-94, attached as Exhibit 7). The use of severe combined immunodeficiency (SCID) mice allows for transplantation of normal and malignant hematological cells (Flavell, 1996, attached as Exhibit 9). The SCID mouse model has also been employed in the art to predict therapeutic benefits of antisense therapy in SCID mice bearing human leukemias and lymphomas (Flavell, 1996, attached as Exhibit 9).

The Gura reference sited by the Examiner states that "animals [xenograft mice] apparently do not handle the drugs exactly the way the human body does." Applicants take this statement to be obvious and irrelevant to this work. It is understood that the mouse model will not **exactly** model the therapeutic results in humans; the mouse experiments are only predictive of outcome in human subjects. The Golden reference states that "most drugs that work in lab

animals turn out to be duds in humans." Applicants do not argue with this generalization, but point to the more pertinent statement of Flavell (1996, attached as Exhibit 9) who writes that "[t]he accumulating experience with the various SCID mouse models of human hematological malignancies indicates that they provide useful and arguably relevant systems in which a range of biologically and clinically relevant problems can be addressed". As this reference uses the same mouse model as the applicant for similar disease states, Applicants believe that this reference is much more relevant than Golden and Gura, both of whom discuss mouse models in general.

Applicants also point the Examiners attention to an example in the specification found on page 41-42, lines 23-3 which teaches of expanding the information from the mouse model to humans with any *bcl-2*-overexpressing cancers.

> This example is concerned with the development of human treatment protocols using the lipid-associated oligo- and polynucleotide compositions. These lipid formulations will be of use in the clinical treatment of various *bcl-2*-overexpressing cancers and diseases in which transformed or cancerous cells play a role. Such treatment will be particularly useful tools in anti-tumor therapy, for example, in treating patients with FL. This treatment will also be useful in treating other conditions that are mediated by *bcl-2* over-expression and resistant to conventional regimens and treatments such as hematologic malignancies, both leukemias and lymphomas, including follicular and nonfollicular lymphomas, chronic lymphocytic leukemia, and plasma cell dyscrasias; solid tumors like those associated with breast, prostate and colon cancer; and immune disorders.

Applicants conclude that in light of the preceding discussion, the specification is enabling for the subject matter claimed, and respectfully request that this rejection be withdrawn.

H. The Claims are Patentable over the Art

The Action asserts that claims 1-9 are rejected under 35 U.S.C. 103(a) as being obvious in light of Evan (WO 93/20200) or Reed (WO 95/08350) or Green *et al.* (U.S. Patent

No. 5,583,034) in view of Tari *et al.* (U.S. Patent No. 5,417,978). Appellants respectfully traverse. The claimed invention is not obvious relative to these references, either alone or in combination.

The Examiner argues that the Evan or Reed or Green *et al.* references teach antisense oligonucleotides targeted to Bcl-2, but do not teach a liposome made of neutral lipids. The Examiner argued that Tari *et al.* teaches an antisense oligonucleotide encapsulated in a liposome, and liposomes comprising phosphatidylcholine or phosphatidylserine, with dioleoylphosphatidycholine preferred. Thus, the Examiner proposed that it would have been obvious to combine an antisense Bcl-2 oligonucleotide of Evan, Reed or Green *et al.* with Tari *et al.*'s liposomes comprising dioleoylphosphatidycholine, with an eye towards improving stability and cellular uptake.

The presently claimed invention is directed to uncharged liposome compositions comprising Bcl-2 related antisense P-ethoxy constructs, and methods of use therefor. Further, none of the cited art teaches P-ethoxy antisense oligonucleotides, and the rejection should be withdrawn on that basis. Regarding the teachings of Tari *et al.*, Appellants disagree with the Examiner's conclusions. At best, this references teaches the benefits of liposomal compositions ***in general***. Tari *et al.* does not provide critical teachings or suggestions that would lead the skilled artisan to adopt neutral, as opposed to charged, liposomes. Thus, the Examiner is improperly interpreting the reference. Further, appellants have demonstrated that charged liposomes, as a class, are toxic, and that uncharged liposomes are non-toxic. Again, appellants find no such teaching in Tari *et al.*, as alleged by the Examiner. As such, appellants respectfully submit that the rejection is based on an improper reading and application of Tari *et al.*

Tari et al. Teaches That All Liposomal Formulations Are Useful

Tari *et al.* clearly teaches the use of liposomal compositions ***broadly***, as described at column 1, line 65 to column 2, line 3:

> The present invention relates to a liposomal methyl phosphonate oligonucleotide composition. The composition comprises (a) a liposome which comprises at least one phospholipid, and (b) an antisense methyl phosphonate oligonucleotide which is entrapped in the liposome.

Tari *et al.* additionally describes liposomes as including various "suitable" phospholipids, at column 3, lines 41-48:

> "Liposomes" is used in this patent to mean lipid-containing vesicles having a lipid bilayer, as well as other lipid carrier particles which can entrap antisense oligonucleotides. The liposomes can be made of one or more phospholipids, optionally including other materials such as sterols. Suitable phospholipids include phosphatidyl cholines, phosphatidyl serines, and many others that are well known in this field.

Tari *et al.* teaches that liposome constructs in general are useful and possess desirable properties, at column 2, lines 49-56:

> The advantages of the invention include improved stability of the antisense oligonucleotides compositions under biologic conditions, improved uptake of the composition in cells, improved incorporation efficiency of the oligonucleotides into liposomes, and enhanced specific therapeutic effect of the antisense oligonucleotides against CML and other disease conditions in which similar gene rearrangements are observed.

Appellants respectfully submit that these teachings point to liposomal compositions generally, and do not identify particular lipids, by class, for use in conjunction with the delivery of oligonucleotides, or any other composition for that matter.

Tari et al. Does Not Point To Neutral Lipids As Being Non-Toxic As A Class

Phoshatidyl cholines are uncharged lipids, and phosphatidyl serines are charged lipids. Tari *et al.* describes phosphatidyl serines, as preferred lipids, and phosphatidyl cholines as preferred or particularly preferred, at column 2, lines 10-14.

> In preferred embodiments of the invention, the at least one phospholipid is selected from the group consisting of phosphatidyl cholines and the phosphatidyl serines, with dioleoyl phosphatidyl choline being a particularly preferred lipid.

One experiment, shown at Table 4, demonstrated that dioleoyl (C18:1) phosphatidylserine, a charged lipid, incorporates MP as well, within statistical error, as the best phospatidylcholine composition tested. The majority of examples described in Tari *et. al.* focused on phosphatidyl choline (PC). As described at column 5, lines 18-22, phoshatidyl choline was selected for use in the majority of experiments incorporating methyl phosphonate oligonucleotides (MP) because:

> (1) both PC and MP are neutral molecules, so they should be compatible and (2) PC is well-studied lipid and easy to handle.

Empty liposomes containing phoshatidyl choline were described as not inhibiting growth in BV173 cells, at column 7, lines 14-16 and 34-36 and at column 7, line 58 to column 8, line 2.

However, Appellants do not find that Tari *et al.* teaches or suggests that the lack of inhibition of growth by phoshatidyl choline was a trait *specific* to phosphatidyl choline, much less to neutral lipids. As described above, Tari *et al.* teaches liposomes in general, and the choice of phosphatidyl choline for the experiments described were based on properties ***other than toxicity***. Appellants find no mention or suggestion in Tari *et al.* that the toxicity of neutral lipids is generally less than other classes such as charged lipids. However, this is the very premise upon which the rejection is based. Its refutation here is strong evidence that the rejection is improper.

The Superior Non-Toxicity Properties of the Claimed Invention over the Compositions of Tari et al. Has Been Demonstrated

Appellants provide a declaration from inventors Tari and Lopez-Berestein (attached as Exhibit D herewith and presented in pending U.S. Application No 08/726,211, filed 10/4/1996). This declaration presents data clearly demonstrating the non-specific toxicity of charged liposomes to the tested cell lines, and the lack of toxicity for uncharged liposomes. This data is surprising and unexpected because it demonstrates the advantage of the presently claimed invention over the teachings of Tari *et al.* of liposomal compositions in general. Such evidence refutes the case for obviousness:

> Office personnel should not require the applicant to show unexpected results over the entire range of properties possessed by a chemical compound or composition. See *e.g.*, *In re Chupp*, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987). Evidence that the compound or composition possesses superior and unexpected properties in one of a spectrum of common properties can be sufficient to rebut a *prima facie* case of obviousness.

M.P.E.P. 2144.08(II)(B).

The specification teaches a selective toxicity to target cells and non-toxicity to other cell types. For example, as demonstrated in the specification at page 7, lines 6-10 and FIG. 1, liposomes comprising neutral lipids and a Bcl-2 oligonucleotide are selectively toxic to cells containing a t(14;18) translocation. This property is further demonstrated in the results showing that liposomes comprising neutral lipids, with or without a non-Bcl-2 oligonucleotide, are relatively non-toxic to either target or control cell lines, at page 7, lines 111-15 and in Fig. 2. The evidence of the non-toxicity of liposomes comprising neutral lipids is further described at page 34, lines 6-10:

> Two control oligonucleotides were used to determine the specificity of the inhibition observed. When L-control oligos or empty liposomes were added to Johnson cells, cell growth inhibition was not observed. Jurkat, Raji and Daudi cells were also treated with L-control oligos and empty liposomes. Non-specific toxicity could be observed when greater than 6 μmol/L of L-OS were used, ***but not with empty liposomes*** (FIG. 2A & FIG. 2B). (Emphasis added)

There is nothing in any of the cited references that would have led the skilled artisan to believe that these results would have flowed *a priori* from the use of liposomes constructed from neutral lipids and Bcl-2 antisense constructs.

The References Posit Insufficient Motivation to Select Neutral Lipids

The Action also fails in supporting the argument that Tari *et al.* provides the proper motivation to combine its teachings with Evan, Reed and Green to specifically select neutral lipids for combination with the oligonucleotides of the claimed invention. The Action argues that the motivation to combine the "liposomal formulation taught by Tari *et al.*" was the teaching

of Tari *et al.* of "antisense oligonucleotides encapsulated in a liposome comprised of neutral lipids, including liposomes composed of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, or, preferably dioleoylphosphatidylcholine." This is a mischaracterization of the teachings of Tari *et al.* Tari *et al.* teaches a "liposomal methyl phosphonate oligonucleotide composition," as described at column 1, line 65 to column 2, line 3:

> The present invention relates to a liposomal methyl phosphonate oligonucleotide composition. The composition comprises (a) a liposome which comprises at least one phospholipid, and (b) an antisense methyl phosphonate oligonucleotide which is entrapped in the liposome.

Further, Tari *et al.* does not disclose liposomes composed of phosphatidylglycerol or phosphatidylethanolamine. The Action attributes the properties of "improved stability and cellular uptake" to dioleoylphosphatidylcholine, when Tari *et al.* teaches that these properties are common to all liposome constructs disclosed in Tari *et al.*, at column 2, lines 49-56:

> The advantages of the invention include improved stability of the antisense oligonucleotides compositions under biologic conditions, improved uptake of the composition in cells, improved incorporation efficiency of the oligonucleotides into liposomes, and enhanced specific therapeutic effect of the antisense oligonucleotides against CML and other disease conditions in which similar gene rearrangements are observed.

Again, it is submitted that the advantages referred to by the Action ***are disclosed in regards to all liposome constructs described by Tari et al.***, without a teaching, suggestion or guidance to use either charged or neutral lipids, let alone specifying the lipid dioleolphosphatidylcholine. Thus, this teaching does not provide the necessary guidance to a neutral lipid to combine this reference with the other cited references.

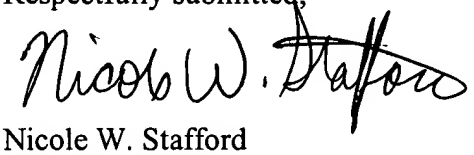
Therefore, appellants respectfully request that this rejection be withdrawn.

I. Conclusions

Applicants have submitted remarks which are believed to place the present claims in condition for allowance. In view of this, Applicants respectfully request that the present claims be passed for allowance. Should the Examiner have any comments or questions with regard to any statements contained herein, or any suggestions as to claim modification, the Examiner is respectfully requested to contact the Applicants' representative listed below at (512) 418-5601.

Please date-stamp and return the enclosed postcard evidencing receipt of these materials.

Respectfully submitted,



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Date: November 10, 2000

APPENDIX 1

1. A composition comprising a P-ethoxy polynucleotide that hybridizes to a Bcl-2-encoding polynucleotide under intracellular conditions and a neutral lipid associated with said P-ethoxy polynucleotide to form a neutrally-charged polynucleotide/lipid association.

2. The composition of claim 1, wherein said P-ethoxy polynucleotide is an oligonucleotide having a length of between about 8 and about 50 bases.

3. The composition of claim 1, wherein the P-ethoxy polynucleotide is complementary to the translation initiation site of Bcl-2 mRNA.

4. The composition of claim 3, wherein the polynucleotide is an oligonucleotide comprising the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1).

5. The composition of claim 1, comprising a liposome formed from the neutral lipid.

6. The composition of claim 5, wherein the P-ethoxy polynucleotide is encapsulated in the liposome.

7. The composition of claim 1, wherein the neutral lipid is a phosphatidylcholine, a phosphatidylglycerol, or a phosphatidylethanolamine.

8. The composition of claim 7, wherein the neutral lipid is dioleoylphosphatidylcholine.

9. A composition comprising an expression construct that encodes a P-ethoxy polynucleotide that hybridizes to a Bcl-2-encoding polynucleotide under intracellular conditions, wherein said P-ethoxy polynucleotide is under the control of a promoter that is capable of expressing in eukaryotic cells, and wherein said construct is associated with a neutral lipid to form a neutrally-charged polynucleotide/lipid association.

10. A method of inhibiting a Bcl-2-associated disease comprising obtaining an antisense polynucleotide that hybridizes to a Bcl-2-encoding polynucleotide under intracellular conditions, mixing the antisense polynucleotide with a neutral lipid to form a polynucleotide/lipid association, and administering said association to a cell, wherein said cell expresses both Bcl-2 and Bax, thereby inhibiting growth of said cell.

11. The method of claim 10, wherein the cell is a cancer cell.

12. The method of claim 11, wherein said cancer cell is a follicular lymphoma cell.

13. The method of claim 10, wherein said P-ethoxy polynucleotide is an oligonucleotide having a length of between about 8 and about 50 bases.

14. The method of claim 10, comprising a liposome formed from the neutral lipid.

15. The method of claim 14, wherein the liposome encapsulates the P-ethoxy polynucleotide.

16. The method of claim 10, wherein said contacting takes place in an animal.

17. The method of claim 16, wherein said animal is a human.

18. The method of claim 17, wherein said association is delivered to said human in a volume of 0.50-10.0 ml per dose.

19. The method of claim 17, wherein said association is delivered to said human in an amount of from about 5 to about 30 mg polynucleotide per m^2.

20. The method of claim 19, wherein said association is administered three times per week for eight weeks.

21. The composition of claim 1, wherein said at least 8 nucleotides are consecutive nucleotides and are targeted to the translation initiation site of Bcl-2 mRNA.

22. The composition of claim 5, wherein said liposome consists essentially of neutral lipids.

23. The composition of claim 9, comprising a liposome formed from said neutral lipid.

24. The composition association of claim 23, wherein said liposome consists essentially of neutral lipids.

25. The composition of claim 10, wherein said antisense polynucleotide is a P-ethoxy polynucleotide.

26. A neutral lipid oligonucleotide association comprising a neutral lipid associated with an antisense oligonucleotide of from about 8 to about 50 bases and complementary to the translation initiation site of Bcl-2 mRNA, wherein said translation initiation site comprises the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1).

27. The neutral lipid oligonucleotide association of claim 26, wherein the oligonucleotide has the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1).

28. The neutral lipid oligonucleotide association of claim 26, comprising a liposome formed from the lipid.

29. The neutral lipid oligonucleotide association of claim 28, wherein the oligonucleotide is encapsulated in the liposome.

30. The neutral lipid oligonucleotide association of claim 28, wherein said liposome consists essentially of neutral lipids.

31. The neutral lipid oligonucleotide association of claim 26, wherein the lipid is a phosphatidylcholine, a phosphatidylglycerol, or a phosphatidylethanolamine.

32. The neutral lipid oligonucleotide association of claim 31, wherein the lipid is dioleoylphosphatidylcholine.

33. A composition comprising a neutral lipid associated with an expression construct that encodes an oligonucleotide of from about 8 to about 50 bases and complementary to at least 8 bases of the translation initiation site of Bcl-2 mRNA, wherein the construct is under the control of a promoter that is capable of expressing peptides in eukaryotic cells.

34. The composition of claim 33, comprising a liposome formed from the lipid.

35. The composition of claim 34, wherein said liposome consists essentially of neutral lipids.

36. A composition comprising a first antisense polynucleotide that hybridizes to a second, Bcl-2-encoding polynucleotide under intracellular conditions and a primary phosphatide associated with said first polynucleotide, wherein said primary phosphatide is a neutral lipid, and wherein said first polynucleotide comprises at least 8 nucleotides of the sequence CAGCGTGCGCCATCCTTC (SEQ ID NO:1), and wherein said polynucleotide is complementary to the translation initiation site of Bcl-2.

37. The composition of claim 36, comprising a liposome formed from the primary phosphatide.

38. The composition of claim 37, wherein said liposome consists essentially of neutral lipids.

39. A method of inhibiting a Bcl-2-associated disease comprising:

a) obtaining an antisense polynucleotide that hybridizes to a Bcl-2-encoding polynucleotide under intracellular conditions;

b) mixing the antisense polynucleotide with a neutral lipid to form a polynucleotide/lipid association; and

c) administering said association to a cell,

wherein said cell expresses both Bcl-2 and Bax, the growth of said cell is inhibited, and the non-specific toxicity of said association is less than the non-specific toxicity of the antisense polynucleotide with DMPC.

40. The method of claim 39, wherein the cell is a cancer cell.

41. The method of claim 40, wherein said cancer cell is a follicular lymphoma cell.

42. The method of claim 39, wherein said polynucleotide is an oligonucleotide having a length of between about 8 and about 50 bases.

43. The method of claim 39, comprising a liposome formed from said neutral lipid.

44. The method of claim 43, wherein the liposome encapsulates said antisense polynucleotide.

45. The method of claim 39, wherein said contacting takes place in an animal.

46. The method of claim 45, wherein said animal is a human.

47. The method of claim 46, wherein said association is delivered to said human in a volume of 0.50-10.0 ml per dose.

48. The method of claim 46, wherein said association is delivered to said human in an amount of from about 5 to about 30 mg polynucleotide per m^2.

49. The method of claim 48, wherein said association is administered three times per week for eight weeks.

50. A method of reducing the non-specific toxicity of a Bcl-2-encoding polynucleotide/lipid association comprising forming a neutrally-charged polynucleotide/lipid association, wherein the non-specific toxicity of said association is less than the non-specific toxicity of the antisense polynucleotide associatied with DMPC.

51. The method of claim 50, wherein the cell is a cancer cell.

52. The method of claim 51, wherein said cancer cell is a follicular lymphoma cell.

53. The method of claim 50, wherein said polynucleotide is an oligonucleotide having a length of between about 8 and about 50 bases.

54. The method of claim 50, comprising a liposome formed from said neutral lipid.

55. The method of claim 54, wherein the liposome encapsulates said antisense polynucleotide.

56. The method of claim 50, wherein said contacting takes place in an animal.

57. The method of claim 56, wherein said animal is a human.

58. The method of claim 57, wherein said association is delivered to said human in a volume of 0.50-10.0 ml per dose.

59. The method of claim 57, wherein said association is delivered to said human in an amount of from about 5 to about 30 mg polynucleotide per m^2.

60. The method of claim 59, wherein said association is administered three times per week for eight weeks.